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Brief report

Drawbacks of the use of cotrimoxazole in foreign-body infections

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ABSTRACT

Introduction: The anti-staphylococcal efficacy of cotrimoxazole in the setting of difficult-to-treat infections seems to be compromised by large amounts of pus and devitalized tissue, and, therefore, high levels of thymidine. Our objective was to evaluate the activity of cotrimoxazole against a staphylococcal foreign-body infection experimental model, which also yields significant quantities of thymidine.
Material and methods: We used a rat tissue-cage model of infection (with high inherent thymidine levels) caused by a strain of methicillin-susceptible *Staphylococcus aureus* (MSSA; ATCC 29213). MIC values were determined (microdilution method) and compared in the presence or absence of tissue-cage fluid samples.
Results: The inefficacy of cotrimoxazole was found to be similar to that of the control group. The MIC of cotrimoxazole was 4–8 fold higher in the presence of rat tissue-cage fluid.
Conclusions: The inefficacy of cotrimoxazole in our foreign-body infection model by MSSA, and the probable negative impact of the presence of thymidine on its efficacy, challenge the use of this drug in acute phases of foreign-body infections. It should be reserved as an alternative treatment when the infection is more controlled.

Inconvenientes del uso de cotrimoxazol en infecciones por cuerpo extraño

Palabras clave:
Cotrimoxazol
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Timidina

RESUMEN

Introducción: La eficacia antiestafilocócica del cotrimoxazol en el marco de las infecciones de difícil tratamiento parece alterarse por la presencia de grandes cantidades de pus y tejido desvitalizado que condicionan unos niveles elevados de timidina. Nuestro objetivo fue evaluar la actividad de cotrimoxazol en un modelo experimental de infección estafilocócica por cuerpo extraño, que también produce grandes cantidades de timidina.
Material y métodos: Utilizamos un modelo de infección de caja subcutánea en rata (con elevados niveles inherentes de timidina) producida por una cepa de *S. aureus* sensible a meticilina (SASM; ATCC29213). Se determinaron los valores de CMI (método de microdilución) y se compararon en presencia del líquido de las cajas o sin él.
Resultados: Cotrimoxazol mostró una ineficacia similar a la de un grupo control. El valor de su CMI aumentó de 4 a 8 veces en presencia del líquido de las cajas.

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Conclusiones: La ineficacia de cotrimoxazol en nuestro modelo de infección de cuerpo extraño por SASM y el probable impacto negativo de la presencia de timidina en su eficacia, cuestionan el uso de este antibiótico en las fases agudas de estas. Por todo ello, cotrimoxazol debería reservarse como tratamiento alternativo cuando la infección esté ya más controlada.

Introduction

High failure rates have been demonstrated in the treatment of serious *Staphylococcus aureus* infections with sulfonamides. Its combination with trimethoprim is synergistic and bactericidal. Consequently, trimethoprim–sulfamethoxazole (TMP–SMZ, cotrimoxazole) has been suggested as an alternative for the treatment of *S. aureus* infections. With regard to its anti-staphylococcal efficacy, previous studies have reported contradictory results, with variable failure rates in certain serious infections.[1] Thus, comparisons of the efficacy of TMP–SMZ, vancomycin and beta-lactams for the treatment of staphylococcal bacteremia and endocarditis found TMP–SMZ to be the least effective.[2] In the setting of osteoarticular infections, some studies have reported good results using TMP–SMZ alone or in combination with rifampicin, whereas other studies have noticed failure rates above 40% and emergence of resistant strains even when using TMP–SMZ at high doses for the treatment of prosthetic joint infections (PJI).[3,4]

A recent review of the use of TMP–SMZ against staphylococcal infections emphasized the importance of the presence of thymidine as a cause of the high failure rates observed in those infections in which large amounts of pus, damaged tissue and bacterial burden are prevalent.[1] In the present study, we aimed to test the *in vivo* efficacy of TMP–SMZ against foreign-body infection by methicillin-susceptible *S. aureus* (MSSA), and to evaluate the extent to which it is affected by the presence of high amounts of thymidine. For these aims, we used a foreign-body infection model in rat, which provides high inherent serum thymidine levels (0.142–0.318 μg/mL).[5–7]

Material and methods

Microorganism and antibiotics

A MSSA strain (ATCC29213) was used for all *in vitro* and *in vivo* studies. Trimethoprim and sulfamethoxazole were purchased from Sigma–Aldrich (Madrid) and Laboratorios Almofarma (Barcelona), respectively.

In vivo *studies*

The experimental protocol complied with European (Directive 2010/63/EU) and Spanish (RD 53/2013) legislation on animal experimentation. The University of Barcelona's Ethics Committee for Animal Experiments approved the animal model previously standardized by our group.[8] Briefly, the methodology consisted of subcutaneous implantation in rats of two Teflon tissue-cages with two polymethylmethacrylate coverslips (CV). After three weeks, the tissue cage fluid (TCF) was infected with MSSA. Three days later, TCF was obtained in order to quantify bacterial counts; therapy was then started and administered subcutaneously for seven days. After the end of treatment, TCF was recovered in order to count bacteria and animals were sacrificed. CVs were removed and processed as previously described.[8] The criterion of efficacy was defined as a decrease in TCF bacterial counts between the beginning and end of treatment, and as the adherent bacteria counts on the CV. The appearance of resistant strains at the end of therapy was screened.

Pharmacokinetic studies

We performed pharmacokinetic studies to select the equivalent human dose of 160 mg TMP/800 mg SMZ every 8 h, which provide the ideal synergistic *in vivo* TMP:SMZ ratio of 1:20 found in human serum. Regarding the features of the tissue-cage infection model, we looked for the appropriate dosage of TMP–SMZ that could guarantee this 1:20 ratio in TCF.

Briefly, the dosage of 120 mg/kg of TMP–SMZ (ratio 1:1, respectively) was subcutaneously administered to animals. Then, blood and TCF samples were collected at 0, 0.5, 1, 2, 4, 6, 8 and 24 h after administration. Samples were centrifuged and serum was transferred into aliquots and stored at −20 °C up to the time of analysis. For the precipitation of serum or TCF protein, trichloroacetic acid solution (50%, 20 μL) was added to 200 μL of serum or TCF samples (calibration and rats) and centrifuged at 18,000 × *g* for 10 min. The supernatants were collected and 5 μL was injected to measure the drug concentration.

The concentrations of TMP and SMZ were analyzed using UHPLC.[9] Chromatography was performed using a Waters Acquity® TM UHPLC system (Waters, MA, USA) with an ultraviolet detector. The separation was carried out with Acquity C18BEH™ (2.1 × 100 mm id, 1.7 μm, Waters, MA, USA). Elution was performed with a mobile phase solution of di-potassium hydrogen phosphate water solution (pH 7.2; 10 mM) containing acetonitrile (20:80). The retention times of TMP and SMZ were 1.4 and 2.7 min respectively. TMP was detected at 270 nm, and SMZ at 254 nm.

In vitro *studies: assessment in the presence of thymidine*

Following the standard procedures, we determined the MIC value of TMP–SMZ (ratio 1:20) using Mueller Hinton Broth (MHB) and microdilution method.

To assess the effect of thymidine on TMP–SMZ activity, we used TCF samples recovered from pharmacokinetic studies (see above), which contained known concentrations of TMP–SMZ and inherent thymidine from rat. The specific thymidine concentration in the TCF samples was not determined, so its possible negative effect on cotrimoxazol efficacy was indirect evaluated by assuming the inherent high concentrations of thymidine in rats.[5] Following the microdilution methodology, we determined the MIC value using a mixture of MHB and this TCF sample (ratio 1:1).

Results

The treatment with TMP–SMZ had to be suspended on the fourth day due to animals presented intolerance to the antibiotic. This therapy did not show significant differences with respect to the control group in terms of efficacy (Table 1). No resistant strains to TMP–SMZ were detected. During treatment with TMP–SMZ the animals showed polyuria and less reactivity. Our hypothesis that

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